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Artemis Pharmaceuticals GmbH 21 May 2001

Claims

1. A recombinant influenza virus for high-yield expression of incorporated foreign gene(s), which is genetically stable in the absence of any helper virus and which has eight viral RNA segments, wherein at least one of the regular viral RNA segments is replaced by an RNA molecule (ambisense RNA segment), said ambisense RNA segment containing one of the standard viral genes in sense orientation and a foreign, recombinant gene in anti-sense orientation, or *vice versa*, covalently linked to each other and in overall convergent arrangement.

2. The recombinant virus according to claim 1, wherein in the ambisense RNA molecule said foreign recombinant gene is covalently bound to one of the viral genes, while the original vRNA segment coding for the same gene is deleted from the recombinant virus by way of specific ribozyme cleavage.

3. The recombinant influenza virus according to claims 1 and 2, wherein one or more of the regular viral RNA segments, differing from said at least one ambisense RNA segment, comprises a vRNA encoding a foreign gene, preferably one or more of the regular viral RNA segments has (have) been exchanged for a vRNA encoding a foreign gene.

4. The recombinant influenza virus according to claim 3 in which one or both of the standard glycoproteins hemagglutinin and neuraminidase have been exchanged into foreign glycoprotein(s) or into fusion glycoproteins consisting of an anchor segment derived from hemagglutinin and an ectodomain obtained from the foreign source, viral or cellular, or in which such recombinant glycoprotein has been inserted as a third molecular species in addition to the remaining standard components.

5. The recombinant influenza virus according to claims 1 to 4, in which the terminal viral RNA sequences of one or more of the regular segments and/or of the at least one ambisense RNA segment, which are active as the promoter signal, have been modified by nucleotide substitutions in up to five positions, resulting in improved transcription rates of both the vRNA promoter as well as the cRNA promoter as present in the complementary sequence.

6. The recombinant influenza virus of claim 5, wherein the 12 nucleotide conserved influenza 3' terminal sequence has been modified by replacement of one to three nucleotides occurring in said sequence at positions 3, 5 and 8 relative to the 3' end by other nucleotides, and/or wherein the 13 nucleotide conserved influenza 5' terminal sequence has been modified by replacement of one or two nucleotides occurring in said sequence at positions 3 and 8 by other nucleotides.

7. The recombinant influenza virus of claim 6, wherein the replacements in the 3' terminal nucleotide sequence comprises the modifications G3A and C8U.

8. The recombinant influenza virus of claim 7, wherein the replacements in the 3' terminal nucleotide sequence comprises the modifications G3A, U5C and C8U, or G3C, U5C and C8G.

9. The recombinant influenza virus of claim 8, which comprises a 3' terminal nucleotide sequence of 5'-CCUGUUUCUACU-3'.

10. The recombinant influenza virus of claims 6 to 9, wherein the 5' terminal nucleotide sequence comprises the modifications U3A and A8U resulting in a 5'-terminal sequence of 5'-AGAAGAAUCAAGG.

11. The recombinant influenza virus according to claims 1 to 10, which is a recombinant influenza A virus.

12. The recombinant influenza virus according to claims 1 to 11, in which the foreign gene(s) in ambisense covalent junction with viral gene(s) code for proteins and/or glycoproteins which are secreted from cells infected with the recombinant virus.

13. The recombinant virus according to claims 1 to 11, in which the foreign gene(s) in ambisense covalent junction with viral gene(s) code for proteins or artificial polypeptides designed to support an efficient presentation of inherent epitopes at the surface of infected cells, for stimulation of a B cell and/or T cell response.

14. A method for the production of recombinant influenza viruses as defined in claims 1 to 13 comprising
(a) RNA polymerase I synthesis of recombinant vRNAs *in vivo,* in ambisense design,
(b) followed by infection with an influenza carrier strain constructed to include flanking ribozyme target sequences in at least one of its viral RNA segments which is (are) to be replaced by the ambisense segments of step (a), and
(c) thereafter selective vRNA inactivation through ribozyme cleavage.

15. A pharmaceutical composition comprising a recombinant influenza virus according to claims 1 to 13.

16. Use of a recombinant influenza virus according to claims 1 to 13 for preparing a medicament for vaccination purposes.

17. The use according to claim 16, wherein the medicament
(a) is suitable against influenza and/or against other infections;

(b) is present in form of inactivated preparations; and/or
(c) is present in form of live recombinant viruses.

18. Use of a recombinant influenza virus according to claims 1 to 13 for preparing agents for somatic gene therapy.

19. Use of a recombinant influenza virus according to claims 1 to 13 for preparing agents, for transfer and expression of foreign genes into cells infected by such viruses.

20. Use of a recombinant influenza virus according to claims 1 to 13 for preparing agents for transfer and expression of RNA molecules into cells infected by such viruses.

21. The use of claim 20, wherein the RNA molecules to be expressed are antisense sequences or double-strand sequences relative to the target cell cellular mRNA molecules, and/or the agent is suitable for sequence-specific gene silencing, preferably by antisense RNA or RNA interference mechanisms.

22. The use according to claims 18 to 21, wherein the agents are applicable in *ex vivo* and *in vivo* application schemes.

23. A method for the production of proteins or glycoproteins which comprises utilizing a recombinant influenza virus according to claims 1 to 13 as expression vector.

24. The method of claim 23, wherein the production is performed in cell culture cells or in fertilized chicken eggs.

25. A method for preventing and/or treating influenza which comprises administering an effective amount of a recombinant influenza virus according to claims 1 to 13 to the mammal to be treated.

26. A method for somatic gene therapy, which method comprises subjecting the organism to be treated with a recombinant influenza virus according to claims 1 to 13.

27. A method for transfer and expression of foreign genes into cells, and for transfer and expression of RNA molecules into cells, which method comprises infecting the cells with a recombinant influenza virus according to claims 1 to 13.

28. Use of a recombinant influenza virus according to claims 1 to 13 for preparing agents for autologous immunotherapy.

29. A method for an immunotherapy which comprises *ex vivo* infection of immune cells with a recombinant influenza virus according to claims 1 to 13, and introduction of the transduced cells into the patient.

30. A method for the induction of antibodies which comprises utilizing a recombinant influenza virus according to claims 1 to 13 as an immunogen.